MULTIPLE ISOFORMS OF FAST AND SLOW TROPONIN-T ARE EXPRESSED IN BOVINE SKELETAL MUSCLES

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Background

Troponin-T (TnT) is well-known for the finding that its degradation products were first observed among numerous skeletal muscle components during postmortem aging of bovine muscles (Olson and Parrish, 1977; MacBride and Parrish, 1977). The degradation of TnT progresses simultaneously with the postmortem tenderization of beef, showing a good correlation between the two events (Penny and Dransfield, 1979). On the other hand, TnT is an important regulatory and structural component of skeletal muscle thin filaments (Perry, 1998). It is also known that there are three distinct TnT genes, namely, fast, slow, and cardiac types, showing the corresponding expression to fast, slow, or cardiac muscle, respectively. Furthermore, fast- and slow-TnT genes generate multiple isoforms by alternative splicing of the mRNAs. Each of all these isoforms appears to be responsible for the specific physiological characteristics required for the various contractile abilities of muscle fiber. Nevertheless, the expression of TnT isoforms in bovine skeletal muscles has not been investigated so far.

Objectives

This study was performed for the purpose of understanding the implications of TnT isoform variation in TnT degradation during aging of bovine muscles. In the present experiment, we analyzed the expression pattern of TnT isoforms among various bovine muscles using RT-PCR and western blotting.

Methods

Various muscles, including the lingual (TN), masseter (MS), pectoralis (PP), diaphragm (DP), psoas major (PM), longissimus thoracis (LT), spinnalis (SP), semitendinosus (ST), semimembranosus (SM), and biceps femoris (BF), were excised from a Holstein cow within 1 hr after slaughter. Total RNA was extracted from each muscle, using ISOGEN (NipponGene, Tokyo, Japan). The first-strand cDNA was synthesized from 0.5 micro gram of total RNA using M-MLV Reverse Transcriptase RNase-H minus (Toyobo, Tokyo, Japan) and 3ADP1 (CTGCAGGAATTCGATATCGAAGCTTGC) as a primer. Then RT-PCR was performed for each muscle using bovine fast- or slow-TnT-specific primers (fast: TGCTGTCCACAGGGAGCTCCAGCC, TTCCGGGATCTTAGGAGCAGTGAGTCTG, slow: GACGCAGCCTAG GCCGCACCAGG, CTGCTCTTCTCCCATGTGGTCGATG). The fast primers were designed for detection of alternative splicing variants. For western blotting analysis, myofibrils were extracted from the MS, DP, and LT by 0.6M KCl, 0.1M potassium phosphate buffer (pH 7.4). The myofibrils were applied to SDS-PAGE using 12.5% gel, followed by western blotting. The TnT bands were detected by using commercially-available anti-fast- or slow-TnT polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA).

Results and discussion

At least four bands of fast TnT isoforms could be detected in the RT-PCR (Fig. 1). The first variant (F1) and the second variant (F2) were commonly observed in all muscles tested. The third variant (F3) appeared to be expressed in mainly faster muscles (PP, PM, LT, SP, ST, SM, and BF), but not in the other three muscles. In contrast, the shortest variant (F4) was exclusively observed in slower muscles (TN, MS, and DP). As for slow type TnT, two isoforms were detected in bovine muscles, the longer one of which (S1) was in all muscles, while the other (S2) was exclusively present in TN, DP, and SP (Fig. 2). Thus, expression patterns of fast- or slow-TnT isoforms varies among skeletal muscles. Taken together, at least four fast- and two slow-TnT isoforms were expressed in bovine skeletal muscles.

Western blotting analysis for MS, DP, and LT showed the differential expression pattern of TnT isoform proteins in muscles (Fig. 3). In LT, two TnT bands were detected, suggesting expression of two fast type isoform proteins, as previously suggested by Huff-Lonergan *et al.* (1996). Negishi *et al.* (1996) also reported that two major bands were detected by an anti-TnT antibody before aging of the muscles. The other bands of fast isoform proteins might not be distinguished by SDS-PAGE analysis due to the low resolution; according to the data of TnT cDNA sequencing (Muroya, unpublished), some of the differences between various splicing products of fast-type isoforms are no more than 5 amino acid residue numbers.

To date, TnT degradation is commonly considered as the indicator of overall postmortem proteolysis and tenderization. According to our unpublished cDNA sequencing data, however, TnT isoforms have distinct cDNA- and amino acid-sequences, suggesting that proteolytic cleavage may occur differently among the isoforms, depending on the amino acid sequences. It may follow that the diversity of TnT isoforms result in variation of the degradation products and of the rate of proteolysis. Therefore, it is important to distinguish which isoform is being expressed and degraded during aging of the muscles.

Conclusions

According to the RT-PCR results, at least four fast-type and two slow-type TnT isoforms appeared to be expressed in bovine skeletal muscles. Two fast- and one slow-TnT isoform proteins were also detected by western blotting analysis. Expression patterns of fast- or slow-TnT isoforms vary among skeletal muscles. These isoforms may undergo different proteolytic cleavages during aging of muscles, depending on the amino acid sequences.

Pertinent literature

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